Comparison of the Ablation and Hyperechoic Zones in Different Tissues Using Microwave and Radio Frequency Ablation

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Objectives—The aim of this study was to compare the differences between the ablation region and hyperechoic zones in microwave and radio frequency ablation of different tissues.

Methods—Microwave and radio frequency ablation were performed on fresh porcine muscle and liver with different power levels for 90 seconds. These 2 ablation methods were then performed on rabbit liver in vivo using 20 W for 60 seconds. The volumes of the ablation and hyperechoic zones were compared following different ablation methods.

Results—The ablation zones were significantly greater than the hyperechoic zones ($P < .05$) with the same power and duration when using 2 ablation methods. The differences of the ablation and hyperechoic zones between muscle and liver tissues were significantly different ($P < .05$). The difference values of the ablation and hyperechoic zones were also significantly different ($P < .05$) using 2 ablation methods.

Conclusions—The hyperechoic zone may have underestimated the extent of ablation using a specified ablation time. In the same tissue, the hyperechoic zone could more accurately estimate the ablation zones using microwave ablation.

Key Words—ablation zone; catheter ablation; microwaves; tissues; ultrasonography

Microwave ablation (MWA) and radio frequency ablation (RFA) are 2 popular thermal ablation techniques in ultrasound-guided treatment for various tumors.[1–3] They are used even more in elderly patients,[4] patients with underlying diseases,[5] or patients with metastatic neoplasms,[6] who are not suitable for surgical treatment[7] and are also used for primary liver tumors,[8] kidney tumors,[9] or other selected tumors (eg, T1a tumors) in selected patients. Although the working principles of RFA and MWA are not exactly the same, the actual ablation zones are both estimated according to the hyperechoic zones under the guidance of ultrasonography. It is therefore helpful to know the relationship between the ablation and hyperechoic zones in tumor ablations. In this study, RFA and MWA were used to treat fresh porcine muscle and liver tissues, respectively, in vitro, and rabbit livers in vivo, to determine the influence of different tissues on the ablation and hyperechoic zones using RFA and MWA, to provide a reference for the treatment of tumors by these forms of ablation.

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Abbreviations

D, difference (value); Dl, longitudinal diameter; Dt, thickness diameter; MWA, microwave ablation; RFA, radio frequency ablation

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Materials and Methods

Materials and Instruments

Fresh porcine muscle (external ridge, a total of 108 pieces, referred to as muscle tissue) and porcine liver (liver tissue, a total of 108 pieces) were purchased from the laboratory of Shengjing Hospital, with an approximate size of 5 cm × 3 cm × 5 cm each. Two New Zealand white male rabbits weighing 3.0 to 3.5 kg each were purchased from the laboratory of Shengjing Hospital and were anesthetized using an intramuscular instillation of 50 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine before each experiment. Booster injections of up to one half of the initial dose were administered as needed. After the anesthesia took effect, the epigastrium of rabbits was shaved. The rabbits were under laparotomy, and the livers of the rabbits were exposed. The rabbits were euthanized by injecting 50 mL of air into the vein after the ablation. The study was approved by the animal care committee of our institute.

A microwave therapy device (ECO-100C; YiGao, Nanjing, China) and its matched cool-tip electrode needle were used. A radio frequency therapy device (WB991029; Olympus, Hamburg, Germany) with a dedicated cold circulation pump (WB950059; Olympus) and a radio frequency cool-tip electrode were also used. An ultrasound instrument (LOGIQ E9; GE, Boston, MA) and ML 6-15 (frequency 4.0–13.0 MHz) were used to check the ultrasound image in the first part of the experiment, while an ultrasound instrument (ESAOTE; MYLAB25; Esaote, Spa, Italy) and LA435 (frequency, 10.0–18.0 MHz) were used in the experiment in vivo. The micrometer (IP54; XiFeng, WuXi, China) with a resolution of 0.01 mm and error of ±0.02 mm was used to measure the ablation zones.

Experimental Methods

The muscle and liver tissues were randomly divided into 3 groups using 15 W, 30 W, and 45 W. Each group included 6 muscle tissues, and 6 liver tissues, so there were 6 muscle and 6 liver samples in the 15W group ($n_{muscle} = 6$, $n_{liver} = 6$), 30 W ($n_{muscle} = 6$, $n_{liver} = 6$), and 45 W ($n_{muscle} = 6$, $n_{liver} = 6$) ablated using RFA. The same distribution of tissues were ablated using MWA. Radio frequency ablation and MWA were used on each group of tissues. Under the guidance of ultrasonography, the ablation electrode needle was inserted into each tissue, followed by 90 seconds of ablation.

Rabbit livers were ablated by RFA and MWA under the guidance of ultrasonography with 20 W for 1 minute, respectively. Twelve lesions were ablated by MWA and RFA in the livers of 2 rabbits.

As much coupling agent as possible was added to the surface of tissue samples to buffer the applied pressure when using the probe to observe the ablation process and measure the hyperechoic range. The ultrasonic probe was mounted as lightly as possible to reduce the pressure on the tissue sample during imaging. At the same time, before measuring the lesion, a sharp cutting knife was used to cut the focal ablation along the long axis of the needle track. After the dissection, a pair of forceps was used to make the section of lesions face up gently. The ablation zone of each tissue was observed, the longitudinal and thickness diameters of the ablations taken from the largest sections were measured, and the volumes of the ablation and hyperechoic zones were calculated. The longitudinal diameter (Dl) and thickness diameter (Dt) were measured, and the volumes of the lesions were calculated as the ellipsoid volume using the formula: ($V = \frac{4}{3}\pi \times \frac{Dl}{2} \times \left(\frac{Dt}{2}\right)^2$) in the experiment. We then compared the difference (D) values of the ablation and hyperechoic zones (D = ablation zone − hyperechoic zone) in both RFA and MWA in different tissues. In the same manner, these experiments were repeated 3 times, and the mean values of the volume measurements were averaged.

Statistical analysis

SPSS statistical software for Windows, version 22.0 (IBM, Armonk, NY) was used for statistical analyses. The data were expressed as $\bar{X} \pm SD$. The independent sample *t* test was used to compare differences between the ablation zones and hyperechoic zones in different tissues and rabbits livers using MWA and RFA and was also used to compare the differences between muscle and liver tissues after ablation using MWA and RFA. The D value of the ablation and hyperechoic zones ablated by these 2 methods in vitro and in rabbit livers was also compared by the independent sample *t* test statistical method. A P value less than 0.05 was considered statistically significant.

Results

Ultrasonography Findings

As seen in the ultrasound images, the ablation zone showed an oval-shaped high-echo zone, with an unclear edge, an irregular shape, and a homogeneous texture. The ablation echo gradually decreased after a few seconds, when the boundary could not be recognized and became similar to that of the surrounding tissues. The hyperechoic zones were measured from the largest sections of the high-echo zones (Figures 1 and 2).

Figure 1. The longitudinal diameter and the thickness diameter of the hyperechoic zone following microwave ablation using 45 W for 90 seconds in liver tissue.

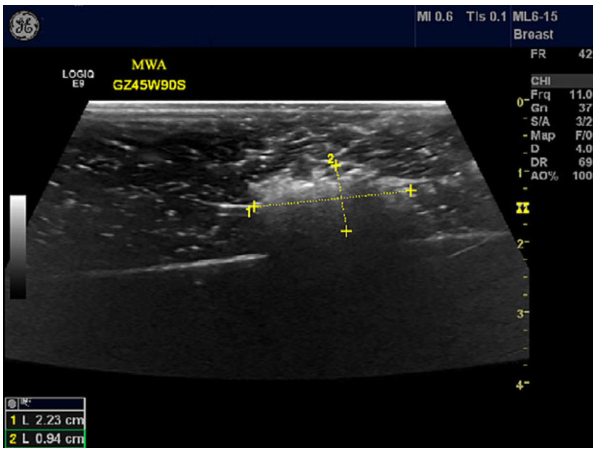
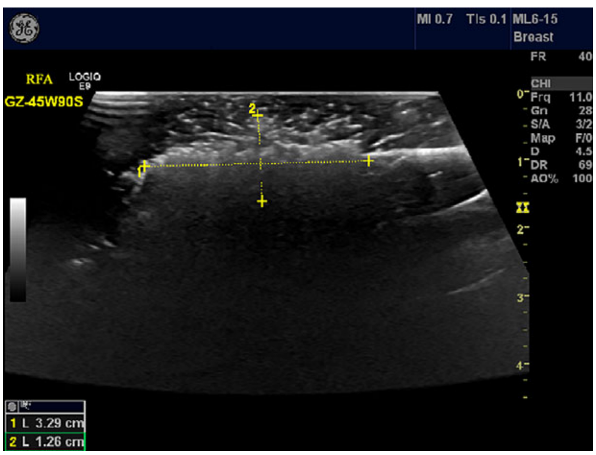


Figure 2. The longitudinal diameter and the thickness diameter of the hyperechoic zone following radio frequency ablation using 45 W for 90 seconds in liver tissue.



Differences Between the Ablation and Hyperechoic Zones in Different Tissues Using RFA and MWA

Microwave ablation and RFA instruments were used to ablate fresh porcine muscle and liver tissues, respectively, using 15 W, 30 W, and 45 W for 90 seconds. After treatment, ablation zones of muscle and liver tissues were evenly distributed and yellowish-white, and the surrounding normal tissue was clearly demarcated, while the ablation zones were wide in the medial section and narrow at both ends (Figures 3 and 4). When muscle tissues were ablated by MWA using 15 W, the ablation zone (516.73 ± 131.10 mm^3) was significantly greater than

Figure 3. The ablation zone following radiofrequency ablation using 45 W for 90 seconds in muscle tissue.

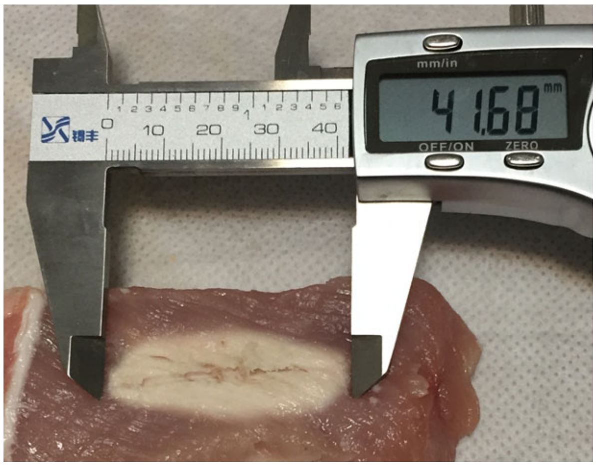
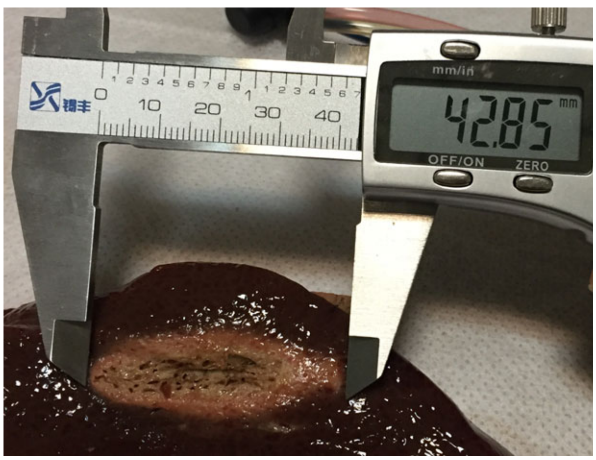


Figure 4. The ablation zone following radiofrequency ablation using 45 W for 90 seconds in liver tissue.



the hyperechoic zone (426.51 ± 126.86 mm^3; P = .043). The ablation zone (766.30 ± 129.46 mm^3) of muscle tissues was also significantly larger than the hyperechoic zone (654.54 ± 165.15 mm^3; P = .030) using 30 W. There was no significant difference between the ablation zones (1,596.42 ± 306.82 mm^3) and hyperechoic zones (1,624.45 ± 335.57 mm^3) in muscle tissue using the power of 45 W (P = .795) (Table 1). The results were similar to that in liver tissues using MWA. When muscle and liver tissues were ablated by RFA using 15 W, 30 W, and 45 W, the ablation zones were significantly larger than the hyperechoic zones (P < .05).

The D Between Muscle and Liver Tissues Treated With MWA and RFA

D_{muscle} and D_{liver} were calculated from the data shown in Table 1. D_{muscle} (90.22 ± 165.83 mm^3) was significantly less than D_{liver} (255.34 ± 102.08 mm^3) using 15 W of MWA (P = .001), and D_{muscle} (111.75 ± 156.65 mm^3) was also significantly less than D_{liver} (286.38 ± 118.44 mm^3) using 30 W of MWA (P = .001), while the D value between the 2 tissues using 45 W was not significant. Radio frequency ablation of muscle and liver tissues using 15 to 45 W for 90 seconds showed that D_{muscle} was significantly larger than D_{liver} (P < .05; Table 2).

Differences of D Determined by MWA and RFA in Muscle and Liver Tissues

Overall, the D value D_{MW} was significantly less than the D_{RF} (P < .05) using most conditions. For example, when muscle tissues were ablated using 15 W, the D_{MW} (90.22 ± 165.83 mm^3) was significantly smaller than that of the D_{RF} (856.48 ± 279.96 mm^3; P < .000). Only when liver tissues were ablated by MWA and RFA for 90 seconds using 15 W was the D value between MWA (255.34 ± 102.08 mm^3) and RFA (361.20 ± 285.39 mm^3) not significant (P = .148; Table 3).

The Differences Between the Ablation and Hyperechoic Zones Using RFA and MWA of Rabbit Livers

The MWA and RFA instruments were used to ablate rabbit livers using 20 W for 90 seconds. After ablation, the ablation zones were pink and evenly

Table 1. Differences Between Ablation and Hyperechoic Zone Volumes in Different Tissues Using MWA and RFA

		Muscle			Liver		
		Ablation Zone (mm^3)	Hyperechoic Zone (mm^3)	*P*	Ablation Zone (mm^3)	Hyperechoic Zone (mm^3)	*P*
MWA	15 W	516.73 ± 131.10	426.51 ± 126.86	.043*	454.27 ± 110.23	198.93 ± 98.05	<.000*
	30 W	766.30 ± 129.46	654.54 ± 165.15	.030*	749.11 ± 115.51	462.73 ± 103.03	<.000*
	45 W	1596.42 ± 306.82	1624.45 ± 335.57	.795	1014.51 ± 245.15	874.0 ± 179.73	.058
RFA	15 W	1253.59 ± 497.11	397.11 ± 217.15	.002*	847.03 ± 279.08	485.83 ± 234.98	<.000*
	30 W	3367.62 ± 1103.66	630.52 ± 198.99	<.000*	3228.09 ± 623.35	1380.47 ± 261.66	<.000*
	45 W	5974.10 ± 694.76	2801.84 ± 833.69	<.000*	4441.14 ± 989.16	2477.16 ± 662.56	<.000*

*P < .05.
Ablation zone: volumes of ablation zone pathologically after the microwave ablation or radiofrequency ablation. Hyperechoic zone: volumes of zone seen from the ultrasonoscopy after the microwave ablation or radiofrequency ablation.
MWA indicates microwave ablation; RFA, radiofrequency ablation.

Table 2. The D Value Between Muscle and Liver Tissues Treated With MWA and RFA

	D Value (MWA)			D Value (RFA)		
	Muscle (mm^3)	Liver (mm^3)	*P*	Muscle (mm^3)	Liver (mm^3)	*P*
15 W	90.22 ± 165.83	255.34 ± 102.08	.001*	856.48 ± 279.96	361.20 ± 285.39	<.000*
30 W	111.75 ± 156.65	286.38 ± 118.44	.001*	2737.10 ± 1059.56	1847.62 ± 519.11	.004*
45 W	–28.02 ± 392.85	140.43 ± 323.80	.169	3172.27 ± 1204.18	1963.98 ± 945.52	.002*

*P < .05.
D indicates the difference value of ablation zone and hyperechoic range; MWA, microwave ablation; RFA, radio frequency ablation.

distributed, which differed from liver tissue in vitro (Figure 5). The shapes of the ablation zones were almost the same, while the surrounding demarcated normal tissue was not as distinct as tissues ablated in vitro. After ablation, there were significant differences between the ablation and hyperechoic zones ($P < .05$); the ablation zones were significantly larger than the hyperechoic zones using both methods (Figure 6).

Comparing the Differences of D Values Between MWA and RFA in Rabbit Livers

When rabbit livers were treated by MWA versus RFA for 1 minute using 20 W, the D value of the ablation and hyperechoic zones showed a significant difference between MWA (171.15 ± 77.50 mm^3) and RFA (517.84 ± 194.86 mm^3) ($P = .001$; Table 4).

Discussion

Ultrasonography is widely used to confirm the size of tumors or to determine if the tumor is benign or malignant.[10] Interventional treatment using ultrasonography is now more commonly used for a favorable prognosis.[11] Although computed tomography, magnetic resonance imaging, elastographic measurements,[12,13] and contrast-enhanced ultrasonography with SonoVue[14] (Bracco International B.V., Amsterdam, the Netherlands) are considered to be more accurate in determining the reduction of ablation after the treatment of ablation, ultrasound imaging using hyperechoic real-time monitoring is still the first choice in estimating the actual extent of ablation during ultrasound-guided ablation therapy.[15] Although the hyperechoic area is related to the lesion,[16] there has been no relevant report on the relationship between the sizes of the ablation and hyperechoic zones. Previous studies have reported that

Figure 5. The ablation zone following radio frequency ablation using 20 W for 60 seconds in rabbit liver.

Figure 6. The longitudinal diameter and the thickness diameter of the hyperechoic region following radio frequency ablation using 20 W for 60 seconds in rabbit liver.

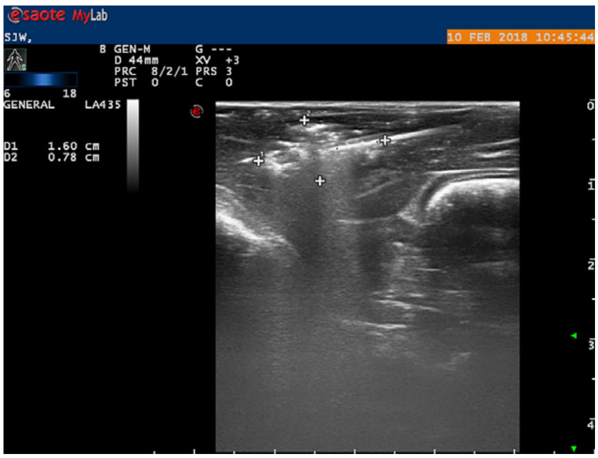


Table 3. The Differences of D Value Determined by MWA and RFA in Muscle and Liver Tissues

	D Value (Muscle)			D Value (Liver)		
	MWA (mm^3)	RFA (mm^3)	*P*	MWA (mm^3)	RFA (mm^3)	*P*
15 W	90.22 ± 165.83	856.48 ± 279.96	<.000*	255.34 ± 102.08	361.20 ± 285.39	.148
30 W	111.75 ± 156.65	2737.10 ± 1059.56	<.000*	286.38 ± 118.44	1847.62 ± 519.11	<.000*
45 W	–28.02 ± 392.85	3172.27 ± 1204.18	<.000*	140.43 ± 323.80	1963.98 ± 945.52	<.000*

*$P < .05$.
D indicates the difference value of ablation zone and hyperechoic range; MWA, microwave ablation; RFA, radio frequency ablation.

local tumor recurrence was significantly higher in large tumors than in small tumors when associated with incomplete ablation of tumor tissue,[17] so the accuracy of ablation treatment is important in determining the effects of treatment as well as in protecting the safety of temperature-sensitive structures near the target lesion.[18] In the present study, we performed MWA and RFA of porcine muscle tissues and liver tissues and rabbit livers and compared the ablation and hyperechoic zones.

In preliminary experiments, we determined that ablation was more effective when we ablated for 90 seconds in different time quantum in vitro. We also found that when using 15 W, the hyperechoic zones started appearing on the ultrasound images after ablating for 60 seconds, so we choose 90 seconds as the optimal duration time for each tissue.

When MWA and RFA were used to ablate muscle and liver tissues for 90 seconds, the ablation zones were significantly larger than the hyperechoic zones under most circumstances for muscle and liver tissues. To a certain extent, because the extent of ablations were reflected by the range of the hyperechoic zones, we could reasonably estimate the ablation areas based on ultrasound images during clinical ablation. However, the range of the hyperechoic zones observed in ultrasound images may have underestimated the ablation zones, which was consistent with the suggestions of Lorentzen et al[19] and Correa-Gallego et al,[20] who showed that both B-mode ultrasound and elastography might underestimate the zones when compared with gross pathologic examinations. Only when the power was 45 W was there no significant difference between the ablation and hyperechoic zones during MWA in both muscle and liver tissues. This may have

Table 4. The Differences of D Value Between MWA and RFA in Rabbit Livers

	MWA	RFA	*P*
Ablation zone (mm^3)	427.38 ± 100.52	763.19 ± 210.45	.005*
Hyperechoic range (mm^3)	252.29 ± 92.16	245.34 ± 148.24	.815
D (mm^3)	171.15 ± 77.50	517.84 ± 194.86	.001*

*$P < .05$.

Ablation zone: volumes of ablation zone pathologically after the microwave ablation or radiofrequency ablation; Hyperechoic zone: volumes of zone seen from the ultrasonoscopy after the microwave ablation or radiofrequency ablation.

D indicates the difference value of ablation zone and hyperechoic range; MWA, microwave ablation; RFA, radio frequency ablation.

resulted from the high initial power, which led to high impedance occurring in the early stage of ablation, with the conduction and heat transfer capacity decreasing and a resulting decrease in heat diffusion.[21] This phenomenon may also be the result of the high power and the prolonged ablation process, resulting in charring before the end of ablation, and carbonization of the high impedance might prevent the outward diffusion of heat,[22] so within a certain range the scope of the ablation and hyperechoic zones increased with the ablation power increased, while there might not be any increases after reaching a certain level.[23,24]

Previous studies have shown that there is a significant difference between ultrasound hyperechoic and ablation zones in vitro.[25] The different values of the hyperechoic and ablation zones were usually greater than zero; therefore, the hyperechoic zone was greater than the ablation zone. In the present study, the results might have some differences because the calculation method of volume in a previous study used the formula ($V = Dl \times Ds \times Dt \times \frac{\pi}{6}$), which measured 3 diameters, including the longitudinal, transverse, and the thickness diameters. However, in the present study, we used the formula ($V = \frac{4}{3}\pi \times \frac{Dl}{2} \times \left(\frac{Dt}{2}\right)^2$). During the ablation process, while the ultrasonic probe was usually used along the ablation needle to observe the longitudinal length and the thickness of the hyperechoic region to ensure real-time monitoring of the ablation, it was difficult to revolve the direction of the probe during clinical treatments to monitor the transverse diameter and to measure it, so by contrast, the Dl and Dt measured in the present study to calculate the volume of the hyperechoic zone might be more consistent with the actual operational requirements. As stated in the research,[26] the ablation lesion was symmetric along the needle path after cutting the lesion, so the hyperechoic zone below the needle could be estimated based on the range in front of the needle. Though the hyperechoic range of the far field may be affected by the sound beam of the needle, the hyperechoic range below the needle could be the same as that in front of the needle on heat conduction. The Dt of the hyperechoic area of the lesion was calculated by multiplying the hyperecho in front of the needle by 2.

The D value of the ablation and hyperechoic zones in muscle tissues using 15 W and 30 W was significantly less than that in liver tissues when MWA was performed. The D values of the ablation and hyperechoic zones in muscle tissues using 15 to 45 W were significantly larger than those in liver tissues when RFA was performed. Muscle tissues might therefore be estimated more accurately than liver tissues when estimating the ablation zone using the hyperechoic zone in MWA, while estimating the ablation zone in liver tissues might be more accurate using RFA. This may be related to the bursting force produced by different ablation methods, as well as the degree of tissue density. Heat generated using microwave involves intermolecular collisions between polar molecules, which are generated by high frequency (approximately 2 GHz) electromagnetic waves. In contrast, heat generated using radio frequency involves an alternating current of approximately 500 kHz that runs from a needle electrode to a metal pad attached to the skin. It is possible that this fundamental difference between MWA and RFA led to the different results, but the specific reason needs further study.[26,27] Sun et al[28] showed a greater tissue penetration depth and a larger ablation zone with a 915-MHz protocol than with the 2,450-MHz protocol due to the longer wavelength. Because of the lower frequency of RFA and its longer wavelengths, it may not have enough bursting force to produce a sufficient hyperechoic effect in muscle tissues when compared with liver tissues. Radio frequency ablation could be a more gentle procedure, and the hyperechoic area of muscle tissues were small, while the penetration depth of the wavelengths might be deeper, so the difference values between ablation and hyperechoic zones were larger than those of MWA. However, the degree of the hyperechoic effects during the ablation burst in liver might be greater than muscle tissue because of the looser components of liver tissue. As previously reported,[29] when the degree of tissue density and water content differ, the range of the hyperechoic zone using ultrasonography may also be different during ablation. The specific characteristics of tissues may result in specific differences in RF-induced coagulation lesions.[30] In ultrasound images, the thyroid tissue is rough, and the nodule is predominantly hard or fibrotic, as in patients with Hashimoto thyroiditis, when compared with normal thyroid tissue. The moisture of thyroid tissues with fibrosis may decrease, so the impedance may be higher and the ablation zone may be reduced, and therefore the maximum volume achieved by ablation may be reduced.[31] Therefore, for treatment of different underlying diseases with MWA or RFA, as a reference for clinical ablation, our data could be used when we estimated the actual ablation zones by observing the hyperechoic zones from ultrasound images.

When MWA and RFA were performed on muscle and liver tissues for 90 seconds using 15 to 45 W, as mentioned above, the different values of the ablation zone versus hyperechoic zone (D) were greater than zero, which meant the extent of the ablation zone was larger than the hyperechoic zone with the same power and duration following MWA and RFA. Difference values following MWA were significantly less than D values following RFA from 15 to 45 W in both tissues, except for D values between MWA and RFA using 15 W in liver tissues. These results indicated that the accuracy of MWA in estimating the ablation zone from the hyperechoic zone was significantly higher than that of RFA when tumors were under thermal ablation.

Considering that there was a lack of blood perfusion, tissue temperature in the living body, and other factors[32] in the in vitro study, we measured the effects of ablation in rabbit livers in vivo, which verified the previously discussed results when estimating the ablation zones from the hyperechoic ranges.

This study had some limitations. Only 12 samples of rabbit livers were ablated, so the results should be further verified in more in vivo experiments, using different types of tissues and more clinical validation, if possible.

In conclusion, when we used the hyperechoic zones to estimate the ablation zones from ultrasound images, the hyperechoic zones we observed may have underestimated the ablation zones, and the accuracy of the estimation in compact tissue (eg, muscle) may have been higher than that in loosened tissue (eg, liver tissue) using MWA, while it may have been lower using RFA. Furthermore, the accuracy using MWA ablation as an estimation method was higher than RFA.

Acknowledgments

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